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Lethal and Sublethal Effects of Methoxyfenozide on the Development, Survival and Reproduction of the Fall Armyworm, *Spodoptera frugiperda* (J. E. Smith) (Lepidoptera: Noctuidae)

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Abstract

The lethal and sublethal effects of the ecdysone agonist methoxyfenozide on the fall armyworm, *Spodoptera frugiperda* (J. E. Smith), were investigated by feeding a methoxyfenozide-treated diet to fifth instars until pupation in doses corresponding to the LC_{10} and LC_{25} for the compound. Larval mortality reached 8% and 26% in the low and high concentration groups, respectively, on the seventh day of the experiment. A progressive larval mortality of 12% for the LC_{10} and 60% for the LC_{25} was observed before pupation. Treated larvae exhibited lower pupal weights, higher pupal mortality, presence of deformed pupae, and more deformed adults than untreated larvae. The incorporation of methoxyfenozide into the diet had a significant effect on the timing of larval development. The development period for males and females was about seven days longer than the controls for both concentrations tested. In contrast, the compound affected neither pupae nor adult longevity. Finally, *S. frugiperda* adults that resulted from fifth instars treated with methoxyfenozide were not affected in their mean cumulative number of eggs laid per female (fecundity), nor percentages of eggs hatched (fertility), or the sex ratio. Our results suggest that the combination of lethal and sublethal effects of methoxyfenozide may have important implications for the population dynamics of the fall armyworm.

Introduction

The compound 20-Hydroxyedysone (20HE) is one of the most active insect ecdysteroid hormones, acting at every stage of the insect's growth to regulate molting and metamorphosis. This hormone plays a crucial role in insect development, and it is theorised that its agonists or antagonists may disrupt the physiological processes of the target pest (Oberlander & Smagghe 2001, Yanagi *et al* 2006). Over the past four decades, efforts have been made to develop insecticides with selective properties that act specifically on biochemical sites that are present in particular insect groups but with properties that differ from other insecticides (Ishaaya *et al* 2005). This

approach has led to the discovery of today's modern insect growth regulator (IGR) insecticides (Dhadialla *et al* 1998).

RH-5849 (the prototype compound) (1,2-dibenzoyl-1-tert-butylhydrazine) tebufenozide, halofenozide, methoxyfenozide, and chromafenozide are all ecdysone agonists (Dhadialla *et al* 1998, Palli & Retnakaran 2001, Yanagi *et al* 2006), a novel class of IGRs (Wing 1988). These compounds mimic the biological function of the natural insect molting hormone 20HE, inducing a premature and lethal larval molt by binding directly to the ecdysteroid receptors (Dhadialla *et al* 1998, Smagghe *et al* 2004). Ecdysone agonists are highly selective against lepidopteran larvae, with the result that other insects are often less affected by them (Silhacek *et al* 1990, Schneider *et al* 2003, 2008). In addition, their narrow spectrum of activity, positive ecotoxicological profile, and short persistence in the environment makes these compounds promising against many economically important agriculture and forest pests (Sundaram *et al* 2002, Smagghe *et al* 2003, Biddinger *et al* 2006, Osorio *et al* 2008).

Like other IGRs, ecdysone agonists act more slowly than neurotoxic insecticides because they disrupt the hormonal system or the physiological development of insects rather than directly killing them (Biddinger *et al* 2006). Although these effects may be important in the field, they have been poorly investigated compared to immediate lethality (Seth *et al* 2004). Since ecdysone agonists do not kill insects immediately, using the criterion of direct mortality caused by ecdysone agonists to assess control efficacy may underestimate their toxicity (Biddinger *et al* 2006).

Studies of the sublethal effects of ecdysone agonists in several important pests have been widely documented, both in larvae and adults. These effects include delayed or accelerated developmental time (Adel & Sehnal 2000, Biddinger *et al* 2006), weight loss of both larvae and pupae (Pineda *et al* 2007, Zamora *et al* 2008), mortality and pupae deformations (Gobbi *et al* 2000, Pineda *et al* 2004), wing deformities in adults (Trisyono & Chippendale 1998, Sundaram *et al* 2002), disturbed diapause (Eizaguirre *et al* 2007), impaired reproductive parameters (Seth *et al* 2004, Smagghe *et al* 2004), and changes in the adult sex ratio (Biddinger *et al* 2006). In addition, Hoelscher & Barret (2003) reported that methoxyfenozide interfered with the ability of *Argyrotaenia velutinana* (Walker) and *Choristoneura rosaceana* (Harris) (Lepidoptera: Tortricidae) adult males to respond to the pheromones of sexually mature females.

In this study, we investigated the lethal and sublethal effects of methoxyfenozide on *S. frugiperda*, the most serious maize pest throughout Latin America, when the larvae were continuously exposed to a methoxyfenozide-treated diet from the fifth instar until pupation.

Material and Methods

Insect rearing

A laboratory colony was started using *S. frugiperda* larvae collected in the summer of 2007 from a maize field at El Trebol, within a 9.5 km radius of the town of Morelia, Michoacan, Mexico. Following collection, the larvae were transported to the Instituto de Investigaciones Agropecuarias y Forestales, Universidad Michoacana de San Nicolas de Hidalgo, Tarimbaro, Michoacan, where all the experiments were performed. The larvae were reared on an artificial diet (Poitout & Bues 1974) in a controlled environmental chamber at 25 ± 2 ºC with 75 ± 5% RH and a photoperiod of 16:8 L:D h. Adults were fed a 15% honey solution. Brown paper was provided as a substrate for oviposition. The paper was replaced periodically.

Bioassays

Newly molted (0-8h) fifth instars of *S. frugiperda* were continuously fed a semi-synthetic diet containing 0.24 mg and 0.35 mg of active ingredient (AI)/kg diet of methoxyfenozide (Intrepid 2 F suspension concentrate, Dow Agrosciences, Zamora, Michoacan, Mexico). We used fifth instars because in other studies we have reported lethal and subethal effects on this developmental stage when ecdysone agonists were applied to different lepidopteran pests (Gobbi *et al* 2000, Sundaram *et al* 2002, Biddinger *et al* 2006). The quantities of methoxyfenozide used correspond to the LC_{10} and LC_{25} values for this insecticide, which were estimated from a preliminary study (Zarate 2008). Both concentrations were prepared in 20 ml water and were mixed into the artificial diet after it had cooled to about 45 ºC but shortly before it solidified.

Groups of 12 larvae were randomly selected. The larvae were individually placed into 2.5-cm^2 cylindrical wells of 12-well Castor tissue culture plates containing approximately 12 g of insecticide-treated diet. Untreated artificial diet was provided for certain specimens as a control. The diet was replaced periodically as necessary until all the larvae had completed the pupal molt. Prior to the bioassay, the larvae were starved for 6h in order to induce a high feeding rate. For the control, CL_{10}, and $CL_{25,}$ we used 168, 384, and 600 larvae, respectively, and 14, 32, and 50 replicates were performed for the respective treatments. Untreated artificial diet was provided to controls.

Bioassays were carried out under the same environmental conditions, as detailed in the section entitled Insect Rearing. The larvae were checked at 24-h intervals for pupation or until mortality occurred. If no movement was observed, larvae were recorded as dead. The larval duration was also determined. The

development of surviving individuals was tracked, and the sublethal effects on pupae and adult emergence were recorded.

To determine pupal weight, between 44-122 male and 38-125 female pupae for each treatment were individually weighed three days after pupation. Afterward, the pupae were individually placed into wells of tissue culture plates as detailed above. After seven days of pupation, the pupae were examined daily for adult emergence, and the adults were classified as normal or deformed. An adult was considered deformed if it was unable to shed from the pupal exuvium or if it did not have normal wings. Pupae were considered dead if an adult did not emerge after 12 d. In order to determine the duration of pupae and adult stages, both were checked daily for mortality. Sex ratio was also assessed.

Effects on reproduction

The sublethal effects of methoxyfenozide were evaluated in specimens derived from larvae that had been exposed to LC_{10} and LC_{25} of insecticide. Pupae were sexed based on examination of the seventh, eighth, and ninth sterno-abdominal segments (Sannino *et al* 1897) by using a stereoscopic microscope (40x) (Zeiss Stemi DV4). After emergence, adults (< 48h old) were placed, using a test tube (2 cm in diameter by 10 cm in height) in a separate oviposition container (7.5 cm in diameter, 5 cm in height) lined with tissue paper. To determine fecundity and fertility, a minimum of 16 and a maximum of 23 pairs of adults were used. They were only provided with a 15% honey solution that was administered using moist cotton and replaced every 2 d to prevent fungal growth. The tissue paper was replaced every 2 d, and fecundity was determined by counting the total number of eggs laid by each female during the first 12 d after the onset of oviposition. Pairs that failed to reproduce were discarded. The percentage of eggs that hatched from those collected 6 d after the first oviposition was used to evaluate fertility. The number of eggs that hatched was assessed 4 d after collection when egg hatching was complete in the control group.

Data analysis

Mortality data for larvae and pupae, pupal formation time, pupal weight, adult emergence time, duration of the larval, pupal, and adult stages, and adult fecundity and fertility were analyzed using one-way ANOVAs. The means were compared by the LSD test ($P < 0.05$) using Statgraphics (Graphic software system; STSC Inc., Rockville) (STSC 1987). In cases where assumptions of the analysis were violated even after performing an arcsine$\sqrt{x}$ or log (x + 1) transformation, a non-parametric Kruskal-Wallis test was applied. The sex ratio in adults was analyzed using the General Linear Model (GLM) procedure with a binomial distribution by sex using SAS Version 8.1 (SAS Institute 2000).

Results

Larval mortality

Fifth instars of *S. frugiperda* that were fed a methoxyfenozide-treated diet exhibited the expected mortality rates for both concentrations when examined seven days after the beginning of the study [(8% for LC_{10} (0.24 mg AI/kg diet) and 26% for LC_{25} (0.35 mg AI/kg diet)] (Table 1). After seven days, we observed a progressive larval mortality of 4% to 34% until just before pupation. Because of the mortality rate after the initial seven days, cumulative larval mortality was 12% for the LC_{10} and 60% for the LC_{25}. Mortality in the control groups did not exceed 5%.

Effects on pupae and adults

Over 70% of larvae that survived the methoxyfenozide treatment exhibited normal pupae (Table 2), which was significantly less than the 100% normal pupae found in the control. The compound also caused a significantly higher percentage of deformed pupae compared with the control (Table 2).

An increase in deformed and normal pupal mortality was observed as the insecticide concentration increased

Table 1 Effects of methoxyfenozide on the larval mortality (mean ± SE) of *Spodoptera frugiperda* when exposed to two concentrations from fifth instar until pupation.

Conc. (mg AI/kg diet)	n	On the seventh day	From the seventh day to pupation	Cumulative
Control	168	0 ± 0 a	3.5 ± 1.14 a	3.5 ± 1.14 a
0.24	384	07.8 ± 1.97 b	4.4 ± 1.05 a	11.9 ± 1.86 b
0.35	600	26.3 ± 1.53 c	34.1 ± 1.61 b	60.4 ± 1.85 c
F		53.42	133.04	141.64
		P < 0.001	P < 0.001	P < 0.001

Within the same column, data followed by the same letter are not significantly different. LSD mean separation; $P > 0.05$; d.f. = 2, 93. n = number of larvae initially tested.

Table 2 Effects of methoxyfenozide (mean ± SE) on pupae resulting from fifth instars of *Spodoptera frugiperda* exposed to two concentrations until pupation.

Conc. (mg AI/kg diet)	Pupae formation (%)[1]		Pupae mortality[1] (%)	N. of specimens dead (%)[2]
	Normal	Deformed		
Control	100.0 ± 0 a	0 ± 0 a	17.9 ± 3.54 a	20.8 ± 3.68 a
0.24	91.7 ± 1.74 b	8.2 ± 1.74 b	26.9 ± 3.27 a	35.1 ± 3.44 b
0.35	71.6 ± 3.09 c	28.3 ± 3.09 c	46.4 ± 3.98 b	78.6 ± 1.80 c
	K = 34.06	K = 34.06	K = 17.91	F = 101.68
	P < 0.001	P < 0.001	P < 0.001	P < 0.001

Within the same column, data followed by the same letter are not significantly different. [1]Kruskal-Wallis, P = 0.05; [2]LSD mean separation, P > 0.05, d.f. = 2, 93).
Pupae mortality includes deformed pupae and normal pupae that had not emerged after 12 days; [2]Combines total mortality during larval and pupal stages.

(Table 2). However, significant differences were only observed at the highest concentration (0.35 mg AI/kg diet), where pupal mortality was 2.6 times higher than the control.

It is important to point out that the combined mortality during larval and pupal stages was higher for both concentrations tested than for untreated larvae (Table 2). At the highest concentration (0.35 mg AI/kg diet), total mortality during these stages was 79%, whereas at the lower concentration (0.24 mg AI/kg diet) mortality was slightly less than half of that figure (35%). Both treatments were significantly different from control group (21%).

Pupae from larvae treated with methoxyfenozide weighed less that untreated larvae, with more pronounced reductions in females than in males (Table 3). At the lower concentration, male pupae underwent a 39% weight reduction while females experienced a 43% reduction. At the higher concentration, males underwent a 47% reduction while females experienced a 50% reduction.

The percentage of normal adults that emerged was reduced across both treatments. To our surprise, the 0.24 mg AI/kg concentration had a lower percentage (88%) of normal adults emerging than the 0.35 mg AI/kg concentration (91%); although small, this difference was significant (Table 3). Significant differences were observed in the rates of emergence of the two concentrations, but both were significantly lower than the control mean of 100%. For the percentage of deformed adults emerging, the opposite trend was observed.

Effects on development

The incorporation of methoxyfenozide into the diet had a significant effect on the duration of the larval stage (Table 4). The larval stage was 7-8 d longer for males and 5-7 d longer for female larvae in both concentrations than in the controls. For both males and females, the pupal stage lasted between 10 and 11 d and was not significantly different from that of the controls (10-11 d).

Neither concentration of methoxyfenozide impacted adult longevity (Table 5). Both male and female adults exhibited identical lifespan (11-12 d) that were not

Table 3 Effects of methoxyfenozide on pupal weights and adult emergence (mean ± SE) in *Spodoptera frugiperda* when exposed to two concentrations as fifth instars until pupation.

Conc. (mg AI/kg diet)	Pupal weight (mg)[1]		Adult formation (%)[2]	
	Male (n)	Female (n)	Normal	Deformed
Control	222.8 ± 4.17aA (44)	221.4 ± 4.85aA (38)	100.0 ± 0.0a	0.0 ± 0.0a
0.24 (CL_{10})	135.3 ± 2.48bA (122)	125.7 ± 1.91bB (125)	88.3 ± 1.69c	11.6 ± 1.69c
0.35 (CL_{25})	118.9 ± 2.69cA (80)	111.3 ± 2.39cB (82)	90.6 ± 2.63b	9.3 ± 2.63b
	F = 237.36	F = 277.30	K = 16.17	K = 16.17
d.f.	2, 243	2, 242		
	P < 0.001	P < 0.001	P = 0.0003	P = 0.0003

Within the same column (lower-case letters) and within rows (upper-case letters), data followed by the same letter are not significantly different. [1]LSD means separation, P > 0.05; [2]Kruskal-Wallis, P = 0.05; n = number of pupae weighed individually.

Table 4 Effects of methoxyfenozide on developmental times (mean ± SE) of *Spodoptera frugiperda* larvae and pupae when exposed to two concentrations as fifth instars until pupation.

Conc. (mg AI/kg diet)	Duration of larval stage (days)		Duration of pupal stage (days)	
	Male (n)	Female (n)	Male (n)	Female (n)
Control	10.4 ± 0.09 a (88)	10.3 ± 0.10 a (74)	10.6 ± 0.18a (71)	9.9 ± 0.16a (59)
0.24 (CL_{10})	17.5 ± 0.26 b (181)	16.7 ± 0.27 b (158)	10.8 ± 0.94a (118)	9.9 ± 0.12a (121)
0.35 (CL_{25})	17.7 ± 0.45 b (111)	15.5 ± 0.34 b (125)	11.2 ± 0.30a (47)	10.8 ± 0.28a (65)
K	189.61	167.59	5.04	11.85
	P < 0.001	P < 0.001	P = 0.080	P = 0.002

Within the same column, data followed by the same letter are not significantly different. Kruskal-Wallis, P = 0.05. n = number of specimens whose development was followed.

significantly different from those of the controls (11 d). In contrast, the lifespan from the fifth instar to adulthood was significantly affected in both sexes (Table 5). Males and females in both treatment groups lived almost twice as long as untreated larvae.

Effects on fecundity and fertility

Adults of *S. frugiperda* derived from fifth instars treated with methoxyfenozide did not exhibit alterations in reproductive parameters. The mean cumulative number of eggs laid per female was 264 for 0.24 mg of AI/kg diet and 356 for 0.35 mg of AI/kg diet. Neither was significantly different from the 393 eggs observed in the control (F = 1.68; d.f = 2, 34; P = 0.20).

Fertility was not affected by either concentration of methoxyfenozide. The percentage of eggs hatched was 95% for the low concentration and 86% for the high concentration. Neither was significantly different from the 85% found in the control (F = 0.80; d.f. = 2, 10; P = 0.48).

Sex ratio

Methoxyfenozide did not appear to impact the adult sex ratio. The male to female ratio for the low concentration treatment was 54:46, while the high concentration treatment group exhibited a ratio of 48:52. Neither was significantly different from the ratio of 54:46 in the control group (F = 1.08; d.f. = 2, 66; P = 0.34)

Discussion

Previous studies demonstrated that ecdysone agonists can cause progressive larval mortality in subsequent instars of the treated insects. These studies also suggested that the chemicals may cause several sublethal effects, such as weight loss in larvae and deformation, in both the pupal and adult stages of surviving individuals. In this study, *S. frugiperda* larvae treated with methoxyfenozide from the fifth instar until pupation showed also a feeding cessation and morphological changes such as double head capsule formation, extrusion of the hindgut, and incapability for shedding the old cuticle. A progressive larval mortality was observed in subsequents instars when early instars of *Spodoptera littoralis* (Boisduval) were treated with methoxyfenozide (Pineda *et al* 2007). Similar results were seen when *Platinota idaeusalis* (Walker) was treated with tebufenozide (Biddinger *et al* 2006). This effect may be due to some combination of a high metabolic stability

Table 5 Effects of methoxyfenozide on development times (mean ± SE) of *Spodoptera frugiperda* adults when exposed to two concentrations as fifth instars until pupation.

Conc. (mg AI/kg diet)	Duration of adult stage (days)		Duration from larvae to adult (days)[1]	
	Male (n)[1]	Female (n)[2]	Male (n)	Female (n)
Control	11.1 ± 0.53 a (71)	11.2 ± 0.44 a (54)	20.7 ± 1.38 a (71)	17.3 ± 0.92 a (59)
0.24	11.0 ± 0.33 a (107)	11.2 ± 0.31 a (99)	38.8 ± 0.49 b (101)	37.0 ± 0.65 b (116)
0.35	11.4 ± 0.74 a (41)	11.8 ± 0.59 a (46)	40.3 ± 0.84 b (44)	38.6 ± 0.95 b (55)
	K = 0.527	F = 0.27	K = 108.70	K = 117.46
	P = 0.768	P = 0.77	P < 0.001	P < 0.001

Within the same column, data followed by the same letter are not significantly different. [1]Kruskal-Wallis, P = 0.05; [2]LSD means separation, P > 0.05; d.f. = 2,196. n = number of specimens whose development was followed.

of the compounds within the larval body tissue and the larval food, and the compounds' high affinities for the target sites. In addition, the long time period over which the compounds were continuously administered may have led to sufficient accumulation of the compound in the larval tissue to induce a lethal molting cycle (Trisyono & Chippendale 1997, 1998).

Spodoptera frugiperda larvae that did not appear to be immediately affected by methoxyfenozide exhibited abnormal or lethal pupation and frequently resulted in larva-pupa intermediates. These effects were also observed in the last instars of *Spodoptera exempta* (Walker), *Spodoptera exigua* (Hübner), *S. littoralis, Mamestra brassicae* (L.), *Galleria mellonella* (L.), and *Mythimna unipuncta* (Haworth) treated topically with RH-5849 or tebufenozide (Budia *et al* 1994, Smagghe & Degheele 1994, Gobbi *et al* 2000). In all these cases molt induction had lethal consequences. The morphological abnormalities occurred because the induction of a rapid molt did not provide enough time for the completion of larval-pupal transformation. Thus, the insects molted to nonviable forms between the life stages (Tateishi *et al* 1993). Molts induced during the early phase of the last instar produce larval-like individuals, while those formed in the late phase generate pupal-like individuals (Eizaguirre *et al* 2007), as was observed in our study.

Larvae that survived the methoxyfenozide treatment experienced high pupal mortality (27% and 46% for LC_{10} and LC_{25}, respectively). Mortality of pupae derived from fourth instars of *S. exigua* and *M. unipuncta* reached 20% and 100%, respectively, when the larvae ingested a low concentration of tebufenozide (0.1 mg AI/kg diet) (Gobbi *et al* 2000). Similarly, when larvae were exposed to the CL_{25} and CL_{50} of this compound as neonates or third instars, the mortality of *P. idaeusalis* pupae was 10-23% for CL_{25} and 22-37% for CL_{50} (Biddinger *et al* 2006). These results, similar to our own, can be explained by the accumulation and persistence of ecdysone agonists in the larval tissue until the pupal molt, at which point the agonist kills the insect. In addition, Sundaram *et al* (2002) reported the presence of an ecdysone receptor complex in the lepidopteran pupae, so it is clear that this life stage is just as susceptible to ecdysone agonists as larval stage.

It is documented that ecdysone agonists cause larvae to stop feeding, what leads to a reduced larval body weight (Pineda *et al* 2006, Eizaguirre *et al* 2007). In our study, the effect of methoxyfenozide on pupal weight was dose-dependent and more pronounced in females than in males. Previously, Biddinger & Hull (1999) reported that pupal weight in both males and females of a susceptible strain of *P. idaeusalis* remained unaffected when neonates were fed tebufenozide at concentrations of CL_{10} and CL_{25}. However, when concentrations of this compound were increased (up to CL_{50}), pupae from treated larvae weighed less than untreated larvae, with more pronounced weight reductions at higher concentrations for females (Biddinger *et al* 2006). As reported by these authors, the major weight reduction in female pupae suggests that females are more susceptible to ecdysone agonists than males. In addition, although a significant difference of deformed adults (2.3%) was observed between high (0.35 mg AI/kg diet) and low (0.24 mg AI/kg diet) concentration, the biological significance of this finding remains unclear.

We observed obvious malformations of the wings in adults that were treated with methoxyfenozide as larvae. Similarly, curled and poorly formed wings were seen in the Lepidoptera *Heliothis zea* (Boddie) (Noctuidae), *Diatraea grandiosella* (Dyar) (Pyralidae), *S. exigua*, *S. littoralis*, and *Choristoneura fumiferana* (Clemens) (Tortricidae) (Chandler *et al* 1992, Trisyono & Chippendale 1998, Carton *et al* 1998, Sundaram *et al* 2002, Pineda *et al* 2004) adults when exposed to tebufenozide, methoxyfenozide or a combination of both compounds as larvae or pupae. It is important to point out that in our study the hind wings were severely affected, and in some cases, they were even vestigial. No abnormalities were noted in the legs, antennae or any other adult body parts. Therefore, it appears that wing development is particularly sensitive to these ecdysone agonists. The affected individuals are functionally dead because the resulting adults are sterile (Sundaram *et al* 2002) or develop in an asynchronous manner to their host plant or normal breeding population (Biddinger *et al* 2006).

Methoxyfenozide caused a delay in the development of *S. frugiperda* larvae when they were exposed as fifth instars. The larval stage lasted 1.6 times longer for treated females and 1.7 times longer for treated males than for untreated larvae. This is very similar to the results seen when larvae of the same insect were exposed as neonates to tebufenozide (Chandler *et al* 1992). This delay increased the total developmental time from larva to adult emergence because treated individuals remained larvae for more than twice as long as the controls (Table 5).

We cannot exclude the possibility that the increase in the length of the larval stage for individuals treated with methoxyfenozide may be due to the fact that larvae in the study had one or two extra molts. For instance, when treated with ecdysone agonists, *Ostrinia nubilalis* (Hübner) (Lepidoptera: Pyralidae) (Trisyono & Chippendale 1997) and *S. littoralis* (Adel & Sehnal 2000) larvae underwent an additional ecdysis into a sixth or seventh instar. A similar study with *S. exigua* resulted in two additional larval molts (Smagghe & Degheele 1994). Unfortunately, this effect was not considered in our work, but additional studies are underway to examine this possibility. The increase in the length of the larval stage for specimens treated with ecdysone agonists is undesirable, because larvae can cause severe damage

to crops. However, larvae receiving sublethal doses may suffer sublethal chronic effects, such as progressive larval mortality, a decrease in pupal weight, pupal mortality, and deformation in both pupae and adults. These effects may be important from a practical standpoint because they might negatively impact pest population dynamics in subsequent generations.

Here we have shown that methoxyfenozide did not have any effect on the longevity of *S. frugiperda* adults as seen in a study of *S. exigua* adults derived from third instars treated with the same compound (Martínez A M, unpublished data). The noctuid moth *Spodoptera litura* (F.) is to our knowledge the only other lepidopteran in which a 50% reduction in adult longevity was observed when exposed as fifth and sixth instars to RH-5849 (Seth *et al* 2004), but the reason for this effect is unclear. Therefore, further research is needed to elucidate the effects of ecdysone agonists on the longevity of adults derived from larvae treated at immature stages.

Most studies of the toxicity of ecdysone agonists on lepidopteran pests have been conducted during the larval stages, and little has been published regarding their effects on reproductive parameters in the surviving individuals. Our study found that methoxyfenozide did not affect the fecundity and fertility of *S. frugiperda* adults resulting from treated larvae, as occurred in *P. idaeusalis* and *D. saccharalis* (Rodríguez *et al* 2001, Biddinger *et al* 2006) adults when exposed to tebufenozide as third instars. In contrast, both reproductive parameters were negatively affected in *S. littoralis* when neonates were exposed to methoxyfenozide (Adel & Shenal 2000). In other studies, a decrease in either the percentage of the eggs hatching (in *H. zea*, Carpenter & Chandler 1994) or the number of eggs laid by the females (in *P. idaeusalis,* Biddinger *et al* 2006) was observed when individuals were treated with tebufenozide as neonates or third instars, respectively. Differences between the reproductive parameters in our study and those reported by other authors may be attributed to the fact that we only exposed the larvae during their fifth and sixth instars, whereas larvae used by Carpenter & Chandler (1994), Adel & Shenal (2000), and Biddinger *et al* (2006) were exposed during their entire larval stage.

Little information is available about the effects of ecdysone agonists on sex ratio. This parameter was not affected in *S. frugiperda* adults. In contrast, Biddinger *et al* (2006) reported a significant change in sex ratio (2:1 male:female) in *P. idaeusalis* adults derived from neonates and third instars exposed to tebufenozide. The mechanisms by which these effects occur are poorly understood. It is reasonable to assume that the differences in sex ratio detected in our study and those reported by Biddinger *et al* (2006) are probably due to a difference in concentrations used (LC_{10} and LC_{25} for *S. frugiperda*; LC_{25} and LC_{50} for *P. idaeusalis*) and the exposure period for the compounds. As reported for *P. idaeusalis,* because of the high mortality during the larval and pupal stages, it was impossible to determine the sex of many of the specimens. This may have rendered any sex ratio change caused by methoxyfenozide unobservable, such as the one seen in *P. idaeusalis* (Biddinger *et al* 2006).

Our studies clearly indicate that LC_{10} and LC_{25} of methoxyfenozide were harmful to *S. frugiperda* larvae. Moreover, survivors showed a great range of delayed sublethal effects such as an increase in larval longevity, a lower pupal weight, a higher pupal mortality and deformed pupae and adults. This is indicative that the combination of lethal and sublethal effects of methoxyfenozide has important implications in the population dynamics of the fall armyworm, contributing to enhance its control.

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